SITE DIRECTED MUTAGENESIS

Thermocycler reaction

Part	Amount
5X Q5 reaction buffer	5 µl
10 µM forward primer	(150 ng) 1 µl
10 µM reverse primer	(150 ng) 1 µl
10 µM dNTPs	0.5 µl
Template DNA	0.8 µl = 53.4 ng (10-60 ng)
Q5 High Fidelity DNA polymerase	0.25 µl
Nuclease-free water	16.45 µl

The reaction mixture was mixed according to the scheme and run in the PCR machine according to the following program:

Step	Temperature	Duration
Initial denaturation	98°C	30 sec
18 X Cycles	98°C 63°C 72°C	30 sec 60 sec 2 min
Final extension	72°C	4 min
Hold	4°C	hold

5 µl of the reaction mixture was mixed with 1 µl of loading dye and run on a 1 % gel together with the plasmid in the initial concentration. The rest of the reaction mixture was treated with 1-2 µl of DpnI for 1 hour at 37°C, heat killed 20 min at 80°C.

The plasmid is then ready for transformation using 5 µl of the mutagenesis product. Selection is done by the introduced restriction site.